Synchrotron X-ray Footprinting of the Gelsolin Segment-1/Actin Complex

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Abstract No. gold9181
Beamline(s): **X28C**

Synchrotron X-Ray footprinting has been developed as a novel technique to investigate the structure of macromolecular complexes in solution and ultimately their dynamics and kinetics. Radiolysis of water by polychromatic synchrotron X-Rays generates hydroxyl radicals that modify specific amino acid side chains within milliseconds. The extent of modification is dependent on both the solvent accessibility and reactivity of the amino acid and can be quantitated by mass spectrometry. This technique is suitable to probe the interface of protein-ligand complexes since the solvent accessibilities of amino acids within the binding interface change in the presence and absence of ligand. A “footprint” of these residues is obtained by comparing their extent of modification in these two states. We have chosen the Gelsolin Segment-1/Actin complex as a model system to develop the method of X-Ray protein footprinting. The crystal structure of this complex has been determined and identifies the amino acids in gelsolin that are involved in binding to actin. We have footprinted the actin-binding helix of gelsolin in the presence of actin. This result is the first X-ray footprint of a protein-protein complex and establishes X-Ray protein footrpinting as a widely applicable technique to probe macromolecular complexes with millisecond resolution.